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DYNAMICS OF TRITIUM ACCUMULATION IN JUVENILE RAINBOW TROUT, SALMO GAIRDNERI, EXPOSED TO TRITIATED WATER, TRITIATED DIET OR BOTH.

Report No 84-217-K

David W. Rodgers
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Resources and Services Unit
Biological Research Section
Chemical Research Department

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ABSTRACT

Tritium dynamics were studied in juvenile rainbow trout, Salmo gairdneri, exposed to tritiated water, tritiated diet or both for 56 d. The activity of tissue water tritium was largely determined by the activity of the ambient water. Tissue water rapidly equilibrated with ambient water, with estimated half lives of less than 30 min. In contrast, the steady state activity of organically bound tritium was significantly affected by the activity of the diet. The steady state activity of organically bound tritium of trout maintained in tritiated water but fed non-tritiated diets was approximately 20% the activity of the ambient water. This steady state activity was achieved relatively quickly (half life approximately 1 d) and likely reflects the exchangeable hydrogen of the test fish. For fish fed tritiated diet, in either tritiated or non-tritiated water, steady state activities of organically bound tritium were in the order of 80% of the activity of the diet. This accumulation was much slower (estimated half lives of 18 - 32 d) and likely represents accumulation of non-exchangeable tritium in the trout.

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	2
RESULTS	6
DISCUSSION	13
SUMMARY	15
ACKNOWLEDGEMENTS	16
REFERENCES	17
APPENDIX 1: Composition of Tritiated Amino Acid Mixture.	19
DISTRIBUTION	- last page -

LIST OF FIGURES

		Page
FIGURE 1:	Specific activity of tissue water tritium and organically bound tritium of rainbow trout during and following exposure to tritiated water.	9
Figure 2:	Specific activity of tissue water tritium and organically bound tritium of rainbow trout during and following exposure to tritiated water and diet.	10
Figure 3:	Specific activity of tissue water tritium and organically bound tritium of rainbow trout during and following exposure to tritiated diet.	11

LIST OF TABLES

		Page
Table 1:	Estimated steady state concentrations and rate constants of tissue water tritium in rainbow trout exposed to tritiated water, diet or both.	7
Table 2:	Estimated steady state concentrations and rate constants of organically bound tritium in rainbow trout exposed to tritiated water, diet or both.	12

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To Mr. F.J. Kee
Director of Research

DYNAMICS OF TRITIUM ACCUMULATION IN JUVENILE RAINBOW TROUT, SALMO GAIRDNERI, EXPOSED TO TRITIATED WATER, TRITIATED DIET OR BOTH

INTRODUCTION

Tritium is one of the major radionuclides released to the environment by Ontario Hydro Nuclear Generating Stations (SSD-AR-80-1) with the majority of tritium released in the form of tritiated water (HTO). In addition, tritium released as tritium gas may be oxidized to tritiated water by a variety of environmental processes (OHRDR 82-525-K). Following release to the environment, tritiated water will be distributed among the components of the ecosystem, including biota.

Within biota, tritium will be distributed in both the tissue water of the organism and its organic material. Organically bound tritium may be further partitioned into an exchangeable fraction, which exchanges hydrogens freely with the ambient tissue water, and a non-exchangeable fraction in which hydrogen-tritium transfer is substantially reduced (Vanderplong et al. 1975). Existing models of the distribution of tritium in the aquatic environment (ie. Vanderplong et al. 1975) are based on the assumption that at steady state, the specific activity of tissue water and organically bound tritium will not exceed the specific activity of the ambient water. This assumption is generally supported by both laboratory experiments and field observations (Patzer et al. 1973; Adams et al. 1979; Blaylock and Frank 1979; Kirchman et al. 1979; Komatsu et al. 1981), however, in a chronically contaminated environment, the specific activity of organically bound tritium has been reported to be as much as twenty times greater than the specific activity of the ambient water (Kirchman et al. 1979).

Because of these uncertainties regarding the behavior of tritium in chronically contaminated environments, the Biological Research Section initiated the Biological Tritium Program. In this Program, laboratory studies have been conducted to define the dynamics of tritium at three trophic levels of an aquatic

ecosystem. Previous studies have concerned the dynamics of tritium in algae, a primary producer (OHRDR 83-383-K) and crayfish, a primary consumer (OHRDR 84-152-K). This report describes the dynamics of tritium in a secondary consumer, rainbow trout, Salmo gairdneri.

In teleosts, there is a fairly rapid exchange of tritium between tissue water and ambient water (Patzer et al. 1973; Kirchman et al. 1979) with steady state levels attained within minutes to hours of exposure. In contrast, a much longer period is required for equilibration of organically bound tritium in the tissues (Patzer et al. 1973; Kirchman et al. 1979; Komatsu et al. 1981). Further, although fish exposed to tritiated water and diets attain higher specific activities of organically bound tritium than fish exposed to tritiated water alone (Patzer et al. 1973; Kirchman et al. 1979; Komatsu et al. 1981), the relative contributions of water and diet to steady state activity, particularly the contribution of diet are poorly defined (Bruner 1973).

In the present study, fingerling rainbow trout were exposed to tritiated water, tritiated diet or both for a period of eight weeks and the dynamics of tissue water tritium and organically bound tritium followed. Tritium exposure was then terminated and the loss of tritium from the fish determined over the next two weeks.

MATERIALS AND METHODS

Juvenile rainbow trout were purchased from a commercial hatchery (Shamrock Springs - Erin, Ont.) and transferred to approximately 400 L tanks supplied with dechlorinated Toronto water. Water temperature was adjusted at the rate of 1 °C/day to 15 ± 1 °C and maintained at this temperature for at least 2 wk prior to experiments. Fish were fed to satiation with a commercial trout diet (GRT-83G, Martin Feed Mills, Elora, Ont.) three times each week. Photoperiod was maintained at 14 h of light throughout the holding period and experiments.

A closed, recirculating water system was employed for tritium exposure experiments to minimize the quantities of tritiated water used. Fish were maintained in circular polyethylene tanks (approximately 50 L) which were contained in large covered polyethylene reservoirs (approximately 450 L). The reservoirs drained by an overflow standpipe into covered polyethylene tanks (approximately 75 L) containing gravel which served as a filter for the recirculating system. Water from the filter was pumped to the fish tanks and produced a current velocity of

approximately 5 cm/s within the tanks. The fish tanks drained by a floor drain (1 cm square mesh) to the reservoir which was maintained at a constant depth by the overflow standpipe. Temperature within the reservoirs was maintained at 15 $\pm$ 1 ° C by circulating chilled water (5°C) through thin walled plastic tubing coiled within each reservoir.

Tritiated water was added to two of the four reservoirs, with the other two reservoirs maintained as non-tritiated controls. Reservoirs to which tritiated water was added each contained four fish holding tanks, while control reservoirs to which tritiated water was not added each contained two fish holding tanks.

Two weeks prior to the introduction of experimental fish, 10 trout were placed in each reservoir. Wastes from these fish provided the nutrients required for the establishment of denitrifying bacteria within the recirculating systems prior to the introduction of experimental fish. When experimental fish were introduced to the reservoirs at an initial density of 80 trout per tank, the initial 'seed' fish were removed from the reservoirs.

Fish were maintained in experimental tanks for two weeks before tritium was introduced to the system. During this interval the trout were fed to satiation once daily for 5 days of the week then not fed the next two days. Trout were then exposed to tritiated water, tritiated diets or both for the next 56 d. Tritiated water was added to two of the four reservoirs at 10:00 of Day 0 to establish a uniform specific activity of approximately 1.5 MBq/L (13.5 kBq/g H) within one hour after addition. The same day, trout in two of the four tanks in reservoirs containing tritiated water and trout in both tanks of control reservoirs were fed their first meals of tritiated diet. The labeled diet contained a mixture of tritiated amino acids and had a specific activity of 1.878 $\pm$ 0.039 MBq/kg. Fish in the other two tanks in reservoirs containing tritiated water were fed non-labeled control diet. Fish were fed daily for 5 days of the week (Monday - Friday) then not fed for the next two days. In each feeding, fish were fed as much food as they would voluntarily consume in a 5-min interval and the quantity of food consumed measured. All fish in each tank were weighed together 'en masse' once each week.

After 56 d, tritiated tanks were flushed and ambient tritium concentrations reduced to less than 2 kBq/L within the first 24 h (background approximately 1 kBq/L). At the same time, fish which had been fed tritiated diets were returned to non-labelled control diet. Fish were fed and maintained as described above for the next 14 d.

Fish were sampled regularly during tritium exposure and following tritium exposure. During tritium exposure fish in tritiated reservoirs were sampled at 0, 0.25, 0.5, 1, 2, 4, 8 and 12 h after addition of tritiated water to the reservoirs. Fish in non-tritiated, control reservoirs were sampled at 0, 1, 2, 8 and 12 h on the first day tritiated diets were fed. Subsequently, fish in all reservoirs were sampled at 1, 2, 4, 7, 14. 21, 28, 35, 42, 49 and 56 d after the start of tritium exposure. Following termination of tritium exposure, trout in reservoirs which had contained tritiated water were sampled at 3 and 5 h after flushing of reservoirs was started. Fish from all reservoirs were then sampled at 1, 2, 3, 7 and 14 d after termination of tritium exposure. For each sample, fish (normally two fish/sample) were removed at random from each tank, killed by cervical dislocation, weighed and frozen (-20 °C) in closed plastic bags until further analysis.

Fish fed actively throughout the experiments, with average voluntary consumption ranging from 3 - 3.5 % wet weight per feeding. Total ammonia concentrations were normally below 0.5 mg/L and were consistently below the limit of 7.4 mg/L which Wedemeyer and Yasutake (1977) suggest as the maximum chronic exposure concentration for cold water fish at experimental temperature (15 °C) and pH (7). Dissolved oxygen concentrations were normally greater than 80% of air saturation, however, on two occasions an interuption of aeration resulted in mortality of some fish. This interuption of air supply resulted in complete mortality of fish in one of the non-tritiated reservoirs after 49 d of exposure to tritiated diets. As surplus fish were present in tanks of the other non-tritiated reservoir, trout were redistributed among the four tanks (two in each reservoir) and the experiment continued for the next 21 d as scheduled. Mortality due to other causes was low and averaged less than 1%/wk over the 10 week experiment. Although the intensity of sampling in some time intervals precluded detailed analysis of growth, the average weight of fish increased from 7.0 $\pm$ 0.2 g (mean $\pm$ SE, 12 tanks) to 15.7 $\pm$ 0.6 g over the course of the 10 wk experiment.

Tritiated water and amino acid mixture were purchased from Amersham, Oakville, Ont. The amino acid mixture consisted of a mixture of 15 L-amino acids including 8 of the 10 amino acids which are essential to trout (NAS 1973). A detailed listing of the composition of the mixture by activity is given in Appendix 1. Tritiated diets were prepared by mixing an aqueous solution containing the required amount of the tritiated amino acid mixture with a powdered commercial diet (GRT-83G). The wet diet mixture was extruded in a food mixer, freeze dried and kept frozen in plastic bags until used. Non-labeled control diet was

frozen in plastic bags until used. Non-labeled control diet was prepared in the same manner but tritiated amino acids were not added.

Tritium activity of water, fish and diet samples was determined by liquid scintilation counting using a Packard Tri-Carb Scintilation Counter (Model 300C). Duplicate samples of reservoir water (1 mL) were mixed with PCS II (14 mL) scintilation cocktail for determination of sample activity. Fish samples were partially thawed, cut into smaller pieces and tissue water extracted by azeotropic distillation with toluene (Moghissi et al. 1973). Duplicate samples of distillate (1 mL) were then mixed with PCS II (14 mL) and tritium activity of tissue water determined. Following extraction of tissue water, samples were desiccated over phosphorus pentoxide to constant weight. Duplicate samples of dried tissue (approximately 0.5 g) were then combusted using a Packard Tri-Carb Oxidizer (Model C306). Combustion water was collected automatically, mixed with Monophase 40 scintilation cocktail (15 mL) and activity of organically bound tritium determined. Distillation of up to 100 g of freeze dried diets (both tritiated and control) did not produce measurable quantities of water and only organically bound tritium activity of these diets was determined. Toluene distillation did not signficantly affect activity of tritiated diet (T-test; $p>0.1$).

As all samples were analyzed within a period of approximately four months, loss of activity due to radioactive decay of tritium (half-life 12.3 y) was not significant. Background activity levels were 39.0 $\pm$ 2.8 DPM/mL (mean $\pm$ SE; n=8) for water, 42.8 $\pm$ 2.8 DPM/mL (n=9) for tissue water and 58.6 $\pm$ 6.9 DPM/g (n=9) for organically bound tritium. (Background organically bound tritium activities were determined by combustion of approximately 0.5 g cellulose as a blank between sample determinations.) Tritium activity of ambient water and tissue water are expressed as specific activity (Bq) per gram of hydrogen. Activity per gram of hydrogen of water samples was obtained by dividing the observed activity by the weight of hydrogen in 1 g of water at 20 C (0.1117 g). Similarly the specific activity of organically bound tritium in fish and diet was obtained by dividing the observed activity by the weight of hydrogen in 1 g (dry weight) of sample.

The hydrogen content of fish and diets was determined gravimetrically after trapping water produced in combustion of oven dried, desiccated samples (OHRDR CE83-2628-K). Hydrogen content of the diets averaged 6.77 $\pm$ 0.05% (mean $\pm$ SE, n=6). The hydrogen content of the fish was similar among fish sampled at the beginning and end of the experiment and averaged 6.91 $\pm$ 0.07% (n=10).

Changes in tritium activity with time were described by a first order, single compartment accumulation model. During the period of tritium exposure (Day 0 - 56), these changes were described by the equation:

$$Q_{(t)} = Q_{(eq)} \quad (1 - e^{-(k1)t}) \qquad (1)$$

where $Q_{(t)}$ is the activity of the sample at time, t, $Q_{(eq)}$ is the steady state (asymptotic) activity attained by the sample, t is time (in days), and k1 is the first order rate constant during tritium exposure.

Changes in tritium activity following termination of tritium exposure, from days 56 to 70 ($56 < t \geq 70$ were described by the equation:

$$Q_{(t)} = Y_{(56)} \quad e^{-k2(t2)} \qquad (2)$$

where $Y_{(56)}$ is the activity of the sample at termination of tritium exposure (day 56) as predicted by equation (1) above, k2 is the first order rate constant during depuration, t2 is time in days following termination of tritium exposure ($t2 = t - 56$) and other terms as given in equation (1). The half life of tritium in either tissue water or organically bound compartments was determined by dividing 0.693 by the estimated rate constant.

The parameters $Q_{(eq)}$, k1 and k2 were estimated simultaneously using an iterative, non-linear least squares procedure (SAS - Proc NLIN). As the model is non-linear, estimated confidence intervals are only approximate. Estimated rates and steady state concentrations were compared using the Z test with differences considered statistically significant if $P < 0.05$ in a two tailed test. Data for individual samples are contained in file No. 837.88.

RESULTS

In tritiated reservoirs, tritium activity of the water increased rapidly after introduction of tritiated water and stabilized at approximately 14 kBq/ g H within the first hour. Mean activities of the reservoirs over the 56 d exposure period were 13.8 $\pm$ 0.07 kBq/g H (mean $\pm$ SE, n=17). In non-tritiated reservoirs, tritium activity was consistently below 10 Bq/g H, which is less than twice background activity. During flushing of tritiated

reservoirs tritium activity decreased more slowly than it increased during addition, however, activity decreased to less than 10 Bq/ g H within the first 24 h and remained at or below this level for the duration of the experiment.

The activity of tissue water tritium of the fish was largely determined by the activity of the ambient water rather than diet (Table 1). For trout in tritiated reservoirs, activity of tissue

TABLE 1

Estimated steady state concentrations and rate constants of tissue water tritium in rainbow trout exposed to tritiated water, diet or both. (Estimate ± SE)

Tritium Source	Steady State Concentration (kBq/g H)	Rate Constant During Exposure (/d)	Rate Constant After Exposure (/d)
Water	13.66 ±0.10	60.20 ± 3.43	7.36 ± 0.43
Food	0.023 ± 0.008	9.27 ± 2.52	0.045 ± 0.014
Both	13.77 ± 0.11	45.91 ± 2.67	5.13 ± 0.31

water tritium increased very rapidly following addition of tritium and decreased rapidly during flushing of the reservoirs, regardless of the tritium activity of the diet (Figures 1 and 2). Similarly, tissue water tritium of fish in the non-tritiated reservoirs remained at low levels during and following the period in which the fish were fed tritiated diet (Figure 3). During tritium exposure, the estimated steady state activity of tissue water tritium of trout in tritiated water alone (13.66 ± 0.10 kBq/g H) was not significantly different from that of trout in tritiated water fed tritiated diets (13.77 ± 0.11 kBq/g H). Although these estimated steady state activities are slightly below the mean specific activity of the ambient water (13.80 ± 0.07 kBq/ g H), these differences were not statistically significant. Among trout in non-tritiated reservoirs, the

estimated steady state activity of tissue water tritium (23 ± 8.0 Bq/g H) was less than 0.2% of the activity of fish in tritiated reservoirs. This steady state activity was only approximately twice the activity of ambient water, even though steady state activity of the organically bound tritium of these fish was approximately 23 kBq/g H.

The rapid exchange of tritiated water between the fish and its ambient water is reflected in the large values of the estimated rate constants, particularly during tritium exposure (Table 1). During tritium exposure, the estimated first order rate constant for accumulation of tritium in tissue water of fish exposed to tritiated water only (60.20 ± 3.43/day) was significantly greater than that of trout exposed to tritiated water and diets (45.91 ± 2.67/day) which in turn was significantly greater than than the estimated rate constant for fish exposed to tritiated diets (9.27 ± 2.52/day). Estimated half lives of tritiated water in tissue water of fish in tritiated reservoirs were 16.6 min for trout exposed to tritiated water and 21.7 min for trout exposed to tritiated water and diets. The first order rate constants estimated after termination of tritium exposure were significantly smaller than rates estimated during tritium exposure likely reflecting the slower flushing of reservoirs during this period.

In contrast to tissue water tritium, accumulation of organically bound tritium was largely controlled by the tritium activity of the diet rather than the water (Table 2). The estimated steady state activity of organically bound tritium of fish in tritiated reservoirs fed non-tritiated diet (2.536 ± 0.095 kBq/g H) was significantly lower than that of trout fed tritiated diets (21.59 ± 1.06 and 23.64 ± 1.75 kBq/g H for fish in tritiated and non-tritiated reservoirs respectively). The estimated steady state activities of organically bound tritium were not significantly different between trout fed tritiated diet in tritiated and non-tritiated reservoirs. Although both estimates of steady state activity of organically bound tritium of trout fed tritiated diets were less than the specific activity of the diet (27.7 ± 2.0 kBq/g H), the estimate of steady state activity of trout in the non-tritiated reservoir was not significantly different, statistically, from the specific activity of the diet.

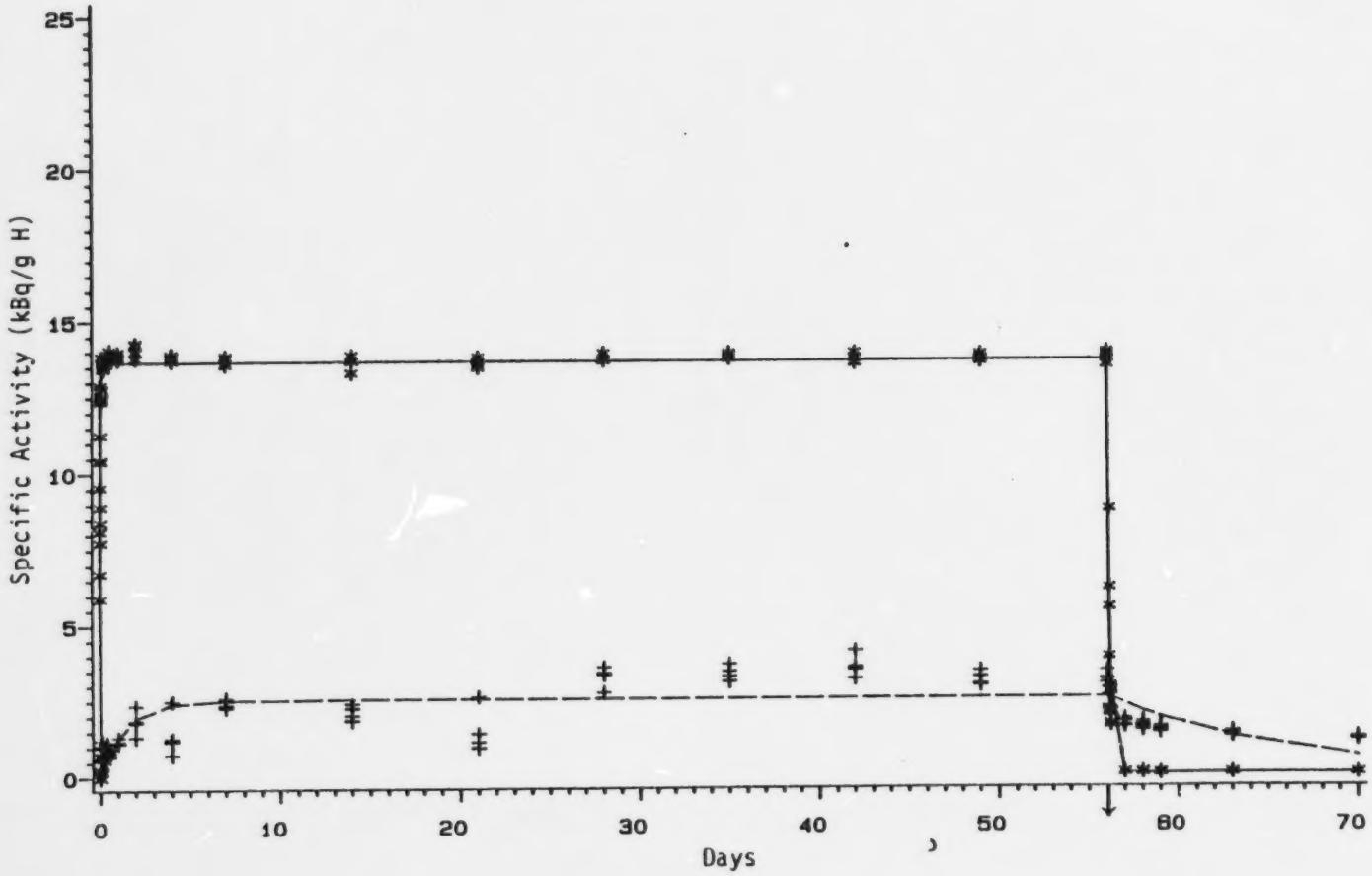

FIGURE 1

SPECIFIC ACTIVITY OF TISSUE WATER TRITIUM (*) AND ORGANICALLY BOUND TRITIUM (+) OF RAINBOW TROUT DURING AND FOLLOWING EXPOSURE TO TRITIATED WATER. TRITIUM EXPOSURE ENDED AT 56d (|)

165574 RD

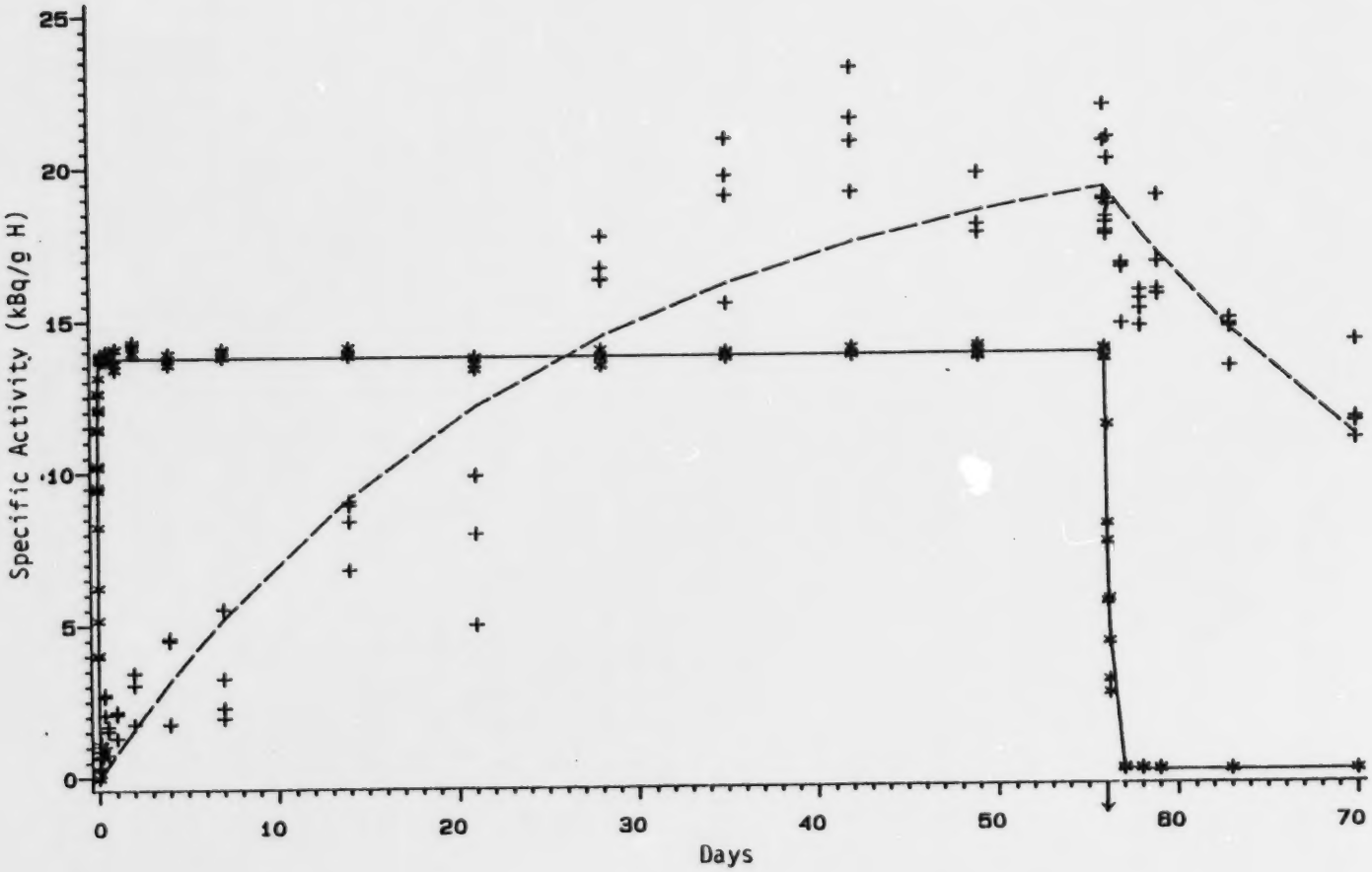

FIGURE 2

SPECIFIC ACTIVITY OF TISSUE WATER TRITIUM (*) AND ORGANICALLY BOUND TRITIUM (+) OF RAINBOW TROUT DURING AND FOLLOWING EXPOSURE TO TRITIATED WATER AND DIET. TRITIUM EXPOSURE ENDED AT 56d (↓)

165575 RD

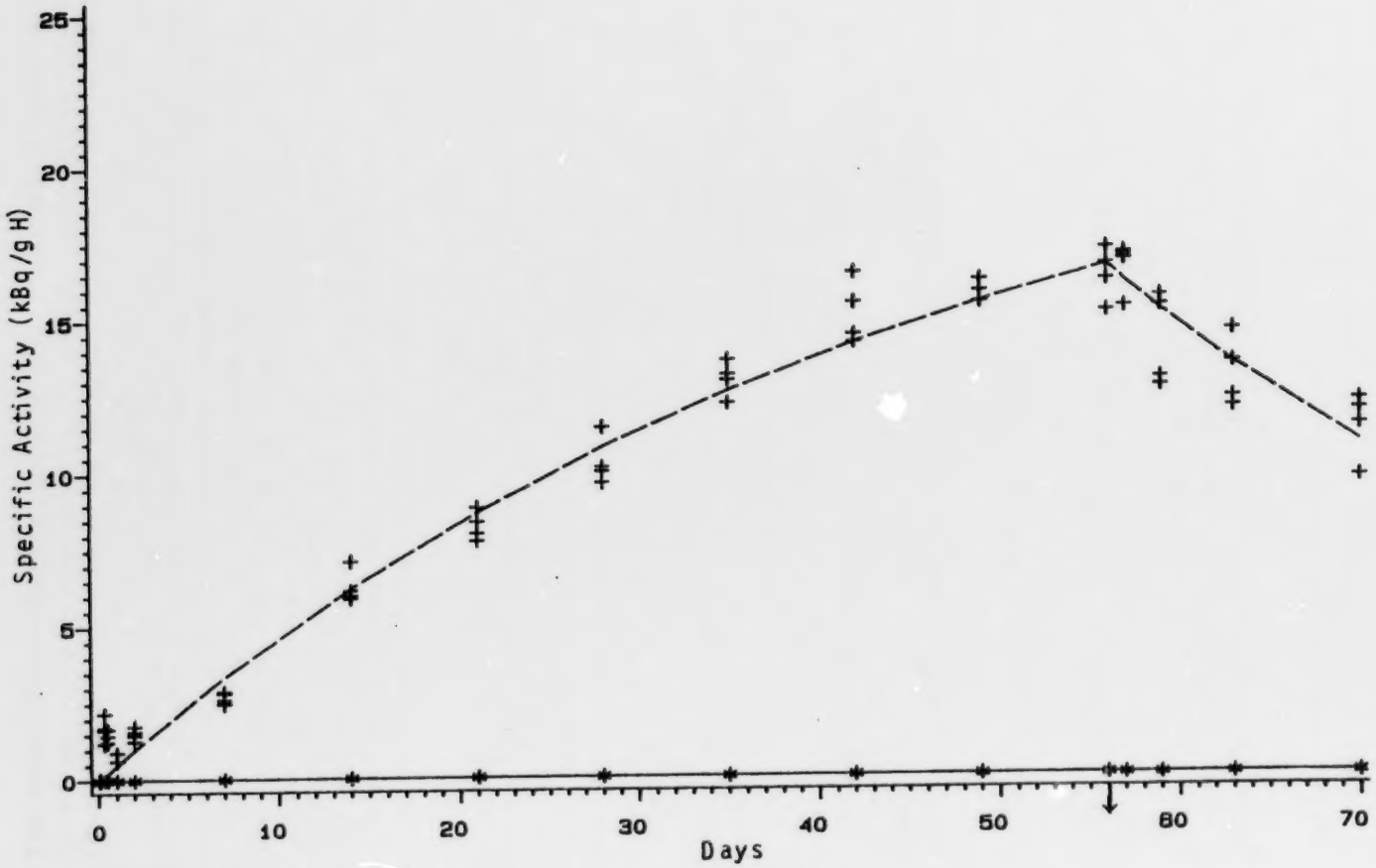

FIGURE 3

SPECIFIC ACTIVITY OF TISSUE WATER TRITIUM (*) AND ORGANICALLY BOUND TRITIUM (+) OF RAINBOW TROUT DURING AND FOLLOWING EXPOSURE TO TRITIATED DIET. TRITIUM EXPOSURE ENDED AT 56d (|)

TABLE 2

Estimated steady state concentrations and rate constants of organically bound tritium in rainbow trout exposed to tritiated water, diet or both. (Estimate ± SE)

Tritium source	Steady State Concentration (kBq/g H)	Rate Constant During Exposure (/d)	Rate Constant After Exposure (/d)
Water	2.536 ± 0.095	0.733 ± 0.158	0.106 ± 0.024
Food	23.65 ± 1.747	.0218 ± .0027	.0308 ± .0031
Both	21.59 ± 1.060	.0393 ± .0049	.0400 ± .0057

The contrast between dynamics of tissue water tritium and organically bound tritium was also apparent in the estimates of rate constants for organically bound tritium, which were markedly lower than the rate constants obtained for tissue water tritium (Table 2). During tritium exposure, the estimated rate constant for organically bound tritium of fish fed tritiated diet in non-tritiated reservoirs (0.0218 ± 0.0027/day) was significantly smaller than that of fish fed tritiated diets in tritiated reservoirs (0.0393 ± 0.0049/day). In turn, both these rate constants were significantly less than that obtained for fish in tritiated reservoirs fed non-tritiated diet (0.733 ± 0.158/day). These rate constants translate into half lives of organically bound tritium of approximately 1 d for fish in tritiated reservoirs fed non-tritiated diets, 17.6 d for fish in tritiated reservoirs fed tritiated diets and 31.8 d for fish fed tritiated diets in non-tritiated reservoirs. Of rate constants estimated for the period following tritium exposure, the estimated rate constant for fish in tritiated water fed non-tritiated diets (0.106 ± 0.024/day)was significantly greater than both the estimated rate constant for fish in tritiated water fed tritiated diets (0.0400 ± 0.0057/day) and the rate constant estimated for fish in non-tritiated water which were fed tritiated diets (0.0308 ± 0.0031/day). These last two rate constants were not significantly different. For fish exposed to tritium via either water or diet alone, the rate constants estimated for the period following tritium exposure were significantly less than rate

constants estimated during tritium exposure. For fish exposed to both tritiated water and diet, the rate constants estimated during and following tritium exposure were not significantly different.

Generally, the estimated rate constants of tissue water tritium were significantly greater than the corresponding rate constant for organically bound tritium. However, for trout fed tritiated diets in non-tritiated water, rate constants of tissue water tritium and organically bound tritium were not significantly different in the period following tritium exposure.

DISCUSSION

The observed dynamics of tritium in rainbow trout resulted from the actions and interactions of three key components. The activity of the tissue water tritium of the fish paralleled that of the ambient water, reflecting the rapid diffusional exchange of water between the fish and its environment. The accumulation of organically bound tritium in the fish reflected the dynamics of two major pools, exchangeable and non-exchangeable tritium. For fish maintained in tritiated water but fed non-tritiated diets, the steady state concentration of organically bound tritium were approximately 20% the activity of the ambient water. These levels were reached relatively rapidly and likely reflect hydrogen-tritium exchange between the tissues of the fish and tissue water. The estimated steady state activity of organically bound tritium in fish fed tritiated diets in either tritiated or non-tritiated water was approximately 80% of the activity of the diet. This accumulation was much slower than that the previous components and likely represents accumulation of non-exchangeable tritium in the tissue of the fish.

The rapid equilibration between tissue water tritium of the trout and ambient water likely reflects the rapid diffusion of water across the gills. The structural and functional properties of the gills which provide for efficient respiratory exchange (large surface area, short diffusion distance and countercurrent flow) also provide for high rates of diffusion of water across the gills (Loretz 1979). The maintenance of steady state specific activities of tissue water tritium which are not significantly different from ambient is consistent with a number of previous studies. (Patzer et al. 1973; Blaylock and Frank 1979; Kirchman et al. 1979; Loretz 1979). Similarly the rapid equilibration of tissue water and ambient tritium as indicated by the small values of the estimated half lives of tissue water tritium is consistent with previous research. For mosquito fish, Gambusia affinis the

half life of tissue water tritium was 14 min (Blaylock and Frank 1979), while the half life of tritiated water in Tilapia mossambica has been reported as 48 min (Kirchman et al. 1979).

The estimated steady state concentration of organically bound tritium in trout maintained in tritiated water but fed non-tritiated diets was approximately 20% of the specific activity of the ambient water. The rapid rate at which this steady state activity was achieved, with an estimated half life for organically bound tritium of approximately one day, indicates that this activity was largely derived from tritium exchange with tissue water rather than metabolic incorporation. The present value of approximately 20% exchangeable organically bound tritium is similar to the values of 20% reported for rainbow trout embryos reared in tritiated water(Bruner 1973), 32% reported for Japanesese killifish, Oryzias latipes grown from larvae to adult in tritiated water but fed non-tritiated diets (Komatsu et al. 1981) and values ranging from 13 to 25% reported for goldfish in aquaria fed commercial diet (Kirchman et al. 1979). Patzer et al. (1973) reported organically bound tritium activities of 40 - 75% of ambient water for a number of species of fish reared in tritiated water and fed commercial diets, however, these fish may have consumed tritiated diets. As approximately 30% of the hydrogens in cellulose are available for tritium exchange (Brown 1979) it is likely that metabolic incorporation of tritium in addition to hydrogen-tritium exchange is required to achieve activities greatly in excess of 30% of ambient water.

For trout fed tritiated diets, the specific activity of the organically bound fraction was primarily determined by diet. Thus the estimated steady state concentrations of organically bound tritium of trout fed tritiated diets in either tritiated or non-tritiated reservoirs were not significantly different, although steady state concentrations were reached faster in fish exposed to both tritiated water and diet. The higher specific activities of organically bound tritium attained by fish in tritiated reservoirs fed tritiated diets relative to fish fed non-tritiated diets is consistent with previous studies (Patzer et al. 1973; Komatsu et al. 1981). Unfortunately, comparable studies of organically bound tritium specific activity of fish fed tritiated diets in non-tritiated water are not presently available. The present results are consistent with studies of Estep and Dabrowski (1980) in which the deuterium to hydrogen ratio of organically bound hydrogen in both mice and marine snails, Littorina obtusata was principally determined by diet.

The longer half life of organically bound tritium relative to tissue water tritium is consistent with previous experimental studies (Elwood 1973; Blaylock and Frank 1979), although previous

studies did not attempt to distinguish between elimination of exchangeable and non-exchangeable organically bound tritium. Interestingly, the half live of organically bound tritium in goldfish was estimated at 8.7 d (Elwood 1973) a value which is intermediate between the values estimated in the present study, of approximately 1 d for presumed exchangeable organically bound tritium and 18-32 d for presumed non-exchangeable tritium. The similarity between half lives of tissue water tritium and organically bound tritium of trout fed tritiated diets in non-tritiated water for the period following tritium exposure may indicate that metabolism or exchange of organically bound tritium of these fish served as the source of tissue water tritium.

In all cases the steady state activity attained by the fish in chronic exposure to tritiated water or diet was less than or equal to the the activity of the source. The present results are thus in agreement with the use of a concentration coefficient of 1.0 in models of the behavior of tritium in aquatic environments (Vanderplong et al. 1975).

These results also demonstrate that fish in non-tritiated water feeding on tritiated food can maintain organically bound tritium activities considerably in excess of the ambient water. This could explain reported instances of fish in natural environments having specific activities of organically bound tritium in excess of the ambient water (Kirchman et al. 1979).

SUMMARY

1. Juvenile rainbow trout were exposed to tritiated water, tritiated diet or both to define tritium dynamics in fish during chronic exposure.

2. The activity of tissue water tritium was essentially controlled by the activity of the ambient water rather than diet. Tissue water rapidly equilibrated with ambient water, with estimated half lives of less than 30 min.

3. The steady state activity of organically bound tritium was significantly affected by diet. The steady state activity of organically bound tritium in trout maintained in tritiated water but fed non-tritiated diets was approximately 20% the activity of the ambient water. This steady state activity was achieved relatively rapidly (half life approximately 1 d) and likely reflects the size of the exchangeable hydrogen pool of the test fish. For fish fed tritiated diet, both in tritiated and non-tritiated water, steady state activities of organically bound

tritium were in the order of 80% of the activity of the diet. This accumulation was much slower (estimated half lives of 18-32 d) and likely represents accumulation of non-exchangeable tritium in the tissue of the fish.

4. In all cases the steady state activity attained by the fish in chronic exposure to tritiated water or diet was less than or equal to the activity of the source. These results are thus in agreement with the use of a concentration coefficient of 1.0 in models of the behaviour of tritium in aquatic environments.

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APPENDIX I

COMPOSITION OF TRITIATED AMINO ACID MIXTURE (AMERSHAM TRK.440-BATCH 38; BATCH ANALYSIS SHEET H/4714)

Amino Acids	Composition by Activity (1 MBq of Mixture Content)	Specific Activity of Component Amino Acids
L-[2,3-^{3}H] Alanine	95 KBq	1.33 TBq/mmoL
L-[5-^{3}H] Arginine (E)	70 KBq	740 GBq/mmoL
L-[2,3-^{3}H] Aspartic Acid	100 KBq	1.26 TBq/mmoL
L-[G-^{3}H] Glutamic Acid	90 KBq	1.41 TBq/mmoL
[2-^{3}H] Glycine	60 KBq	459 GBq/mmoL
L-[2,5-^{3}H] Histidine (E)	15 KBq	2.07 TBq/mmoL
L-[4,5-^{3}H] Isoleucine (E)	60 KBq	4.44 TBq/mmoL
L-[4,5-^{3}H] Leucine (E)	125 KBq	2.37 TBq/mmoL
L-[4,5-^{3}H] Lysine (E)	50 KBq	1.48 TBq/mmoL
L-[4-^{3}H] Phenylalanine (E)	75 KBq	925 GBq/mmoL
L-[3,4(n)-^{3}H] Proline	55 KBq	1.48 TBq/mmoL
L-[3-^{3}H] Serine	30 KBq	481 GBq/mmoL
L-[G-^{3}H] Theronine* (E)	55 KBq	8.51 GBq/mmoL
L-[G-^{3}H] Tyrosine	60 KBq	2.15 TBq/mmoL
L-[3,4(n)-^{3}H] Valine (E)	60 KBq	1.18 TBq/mmoL

E - Essential Amino Acid for Trout (NAS 1973)

* - Double Quantity Supplied as Compund Present as DL - amino acids